The Virtual Institute of Microbial Stress and Survival

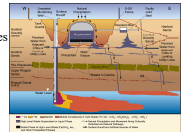
Rapid Deduction of Stress Response Pathways in Metal/Radionuclide Reducing Bacteria

Adam P. Arkin[1,2] and Terry C. Hazen[1] PI

Jay Keasling[2], Anup Singh[7], Frank Olkin[1], Inna Dubchak[1],Hoi-Ying Holman[1], David Stahl[3], Matthew Fields[4], Dorothea Thompson[4], Jizhong Zhou[4], Judy Wall[5], Martin Keller[6]

[1]Lawrence Berkeley National Lab, [2]University of California-Berkeley, [3]University of Washington, [4]Oak Ridge National Lab, [5]University of Missouri, [6]Diversa Inc., [7]Sandia National Lab

Overview

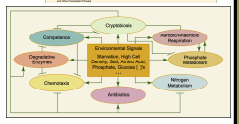


The Problem

- DOE oversees 350 cleanup projects involving soil contaminated with metals or radionuclides, Life-cycle cost $220 billion over 70 years, could be $300 billion without breakthroughs
- Bacteria can immobilize and detoxify metals in soil via reduction to less soluble and less toxic forms, this occurs naturally and be stimulated in situ.
- A thorough understanding of the biogeochemistry, especially stress responses in metal/radionuclide bacteria, will enable prediction of natural attenuation and new strategies for remediation that could save DOE billions in cleanup, risk assessment, and environmental stewardship.

Overarching Goal

- Develop criteria for monitoring the integrity (health) and altering the trajectory of an environmental biological system (process control)
  - Requires a complete understanding of how the biological "units" comprising the system are organized, regulated, and linked in time and space (genes, genomes, cells, populations, communities, and ultimately, ecosystems)
  - Key to these objectives is a more complete understanding of stress response systems and their environmental context

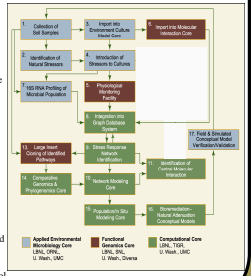


Approach

- Large-scale comparative systems biology of three target bacteria known to be involved in these processes.
  - *Shewanella oneidensis*- with help from the Shewanella Federation
  - *Geobacter metallireducens*- with help from the Geobacter Project
  - *Desulfovibrio vulgaris*: our main focus.
- Field sampling of strains present at DOE waste sites, characterization of in situ physiology, and the stressors that prevent optimal reduction of metals.
- High-throughput functional genomics (RNA, proteins, metabolites, molecular interactions) to identify pathways that respond to the different stressors.
- Apply comparative genomics and data analytical approaches to deduce models of the pathway topologies and dynamics suitable for predicting cellular response to the stressors.
- Use of the models to explore different protocols for stimulating bacteria to reduce metals more efficiently and for accurately predicting natural attenuation strategies, where they are applicable.

Organization

- The effort is organized into three cores each providing general purpose facilities applied to our target organisms
  - Applied Environmental Microbiology Core (AEMC): Field collection, soil simulation, in situ physiological monitoring, community characterization, generation of biomass for FGC.
  - Functional Genomics Core (FGC): Transcriptome, proteome, metabolome and interaction measurements.
  - Computational core (CC): Data management and serving, data analysis, network deduction, pathway modeling, and conceptual biostimulatory protocol development with AEMC.



Applied Environmental Microbiology Core

1. Make biologically defensible environmental simulators
2. Measure and simulate soil stressors
3. Collect information on the soil distribution of different species and their growth under different conditions
4. Produce biomass for testing by FGC
5. Test conceptual models in lab and field

Functional Genomics Core

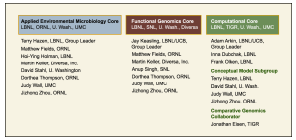
1. Identify response pathways using microarrays, proteomics and metabolomics
2. Experimentally measure and computationally predict cis-regulatory and protein-protein interactions within the networks of interest
3. Employ perturbation response experiments to deduce previously unobserved network structure
4. Clone out homologous pathways from nearby microbes and use comparative analysis to further refine network predictions
5. Compare results within and across niches to generate hypothetical "teleologies" for the network structure

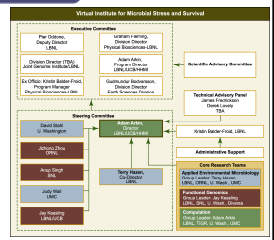
Computational Core

1. Store information in pathway context
2. Serve data and analyses back to experimentalists
3. Develop models of the key organisms
4. Validate models against molecular profiling and growth data
5. Use models as basis for conceptual model of attenuation and bioremediation
6. Analyze combined model to produce policies for bioremediation
7. Re-entry into model improvement cycle

http://vimss.lbl.gov

Organization





Overall Deliverables

1. High-throughput deduction of biomolecules in stress response and data analyses
2. New technologies for observing microbial diversity and cloning homologous pathways
3. Working computational models for stress response behavior, life cycle and evolution
4. Documentation of natural attenuation; optimized strategies for bioimmobilization

Experimental Core Facilities

Applied Environmental Microbiology Core Facilities

Environmental Molecular Microbiology Facility (ORNL, LBNL, U. Wash., Diversa Inc.):
Environmental Simulation and Culture Facility (LBNL, U. Wash., Diversa Inc., U. Missouri):

Computational Core Facilities: (UCB, LBNL)

Stress Response Pathway Database (B-SaSS)
Comparative Genomics Pipeline (C-GASSP)
Data analysis and Reverse Engineering Pipeline (DARE-SSP)
Comparative Modeling of Stress Response Tools (MoSSPath)

Functional Genomics Core Facilities

Metabolite/Protein Profiling Facility (UCB)
Microbial Genomics Facility (ORNL)